PATENT
Docket No.: 4167-4000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants	:	Bradshaw, M. et al	Group Art Unit: To be assigned
Serial No.	:	To be assigned	Examiner: To be assigned
Filed	:	Herewith	
For	:	A NEW YEAST-BACTERIA SHUTTLE VECTOR	

PRELIMINARY AMENDMENT

Commissioner For Patents
Washington, D.C. 20231

Dear Sir:

Please amend the claims as follows.

IN THE CLAIMS

Please cancel claim 1 and add the following claims.

14. A method of cloning, manipulating and performing mutagenesis of defined segment of DNA having flanking sequences using site-specific targeting comprising:

a. obtaining a targeting vector comprising a yeast-bacteria shuttle vector comprising a yeast replication origin, a yeast selection marker gene, a bacterial replication origin, a bacterial selection marker gene, at least one unique cloning site, and sequences homologous to the sequences flanking the defined segment of DNA, wherein said bacterial replication origin maintains a single copy of the targeting vector within a bacterial host;

b. linearizing the targeting vector within the homologous sequences to form recombinogenic ends;

c. introducing the linearized targeting vector into a yeast cell containing DNA comprising the defined segment of DNA;

d. performing mutagenesis of the defined segment of DNA; and

e. selecting for a recombinant product containing the defined segment of DNA.

15. The method of claim 14, wherein said bacterial replication origin is selected from the group consisting of P1 replicon and F factor origin of replication.

16. The method of claim 14, wherein the defined segment of DNA is mutated by yeast genetics.

17. The method of claim 14, wherein the defined segment of DNA is mutated in bacteria.

18. The method of claim 15, further comprising the step of using the defined segment of DNA to create knock-in or knock-out strains of mammals.

19. The method of claim 15, further comprising the step of using the defined segment of DNA to create transgenic embryos.

20. The method of claim 14, further comprising manipulating the recombinant product by

mutagenesis of the defined segment of DNA by homologous recombination in yeast.

REMARKS

Support for new claims 14, 16 and 17 is found in the specification as filed, for example, in original claim 14; Examples 2 and 3; page 6, lines 21-24; at page 3, lines 8-17; page 7, lines 18-21; and at page 12, lines 2-13; and elsewhere throughout the application. Support for new claim 15 is found, inter alia, in the specification at page 6, lines 21-24; and elsewhere throughout the application. Support for new claim 18 is found, inter alia, in the specification at page 13, lines 8-20, and support for new claim 19 is found, inter alia, in the specification at page 12, lines 2-13. Support for new claim 20 is found, *inter alia*, at page 3, lines 10-13. No new matter is introduced by the new claims.

Respectfully submitted,

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